TruePrime™ Liquid Biopsy: the first tool to amplify cell-free DNA

Angel J. Picher[1], Raquel Juárez[1], Bettina Budeus[2], Laura Muinelo[3], Beatriz Sobrino[3], Rafael López[3], Vanesa García-Barberán[4], Patricia LLovet[4], Trinidad Caldés[4]

[1]Expedeon SLU, Madrid, Spain [2]Expedeon Bioscience GmbH & Co KG, Heidelberg, Germany [3]Fundación Ramón Domínguez, Complejo Hospitalario Santiago de Compostela, Spain [4]Hospital Clínico San Carlos, Madrid, Spain

Abstract

TruePrime™ is the name of a novel multiple displacement amplification (MDA) technology initially dedicated to the amplification of genomic DNA (Picher et al, Nat. Commun. 2016). While the current gold standard MDA relies on random synthetic primers to start off the amplification, TruePrime™ is based on a combination of Phi29 DNA polymerase with the recently discovered primase/polymerase TthPrimPol. In this setup, TthPrimPol synthesizes the DNA primers needed for Phi29 DNA pol in the course of the reaction, which allows for the exponential amplification of genomic DNA. Key advantages of the TruePrime™ technology include complete absence of primer artefacts, higher sensitivity down to the femtogram range, high reproducibility, little bias in genome coverage, and superior variant detection.

The TruePrime apoptotic cell-free DNA amplification kit combines the novel TruePrime DNA amplification technology, with key novel steps of cell-free DNA pretreatment, composed of an end-repair + dA tailing reaction and ligation of hairpin-adaptors. This pretreatment enables the efficient amplification of apoptotic cell-free DNA by TruePrime following the rolling circle DNA amplification method, in which TthPrimPol generates primers on the hairpin-adaptors that are extended by Phi29 DNA pol. The strong strand displacement capacity of Phi29 DNA polymerase allows TthPrimPol to synthesize new primers on the new hairpins generated, resulting in exponential isothermal DNA amplification.

Results on the amplification and sequencing using different approaches (whole genome and targeted sequencing) and platforms (Illumina and Ion Torrent) will be presented comparing the data obtained from amplified cell-free DNAsamples from cancer patients and the corresponding non-amplified cell-free DNA, demonstrating the usefulness of TruePrime LiquidBiopsy technology for cell-free DNA analysis.

We have adapted the TruePrime technology towards the amplification of cell-free DNA from liquid biopsies with the intention toimprove sensitivity and reliability of the method, resulting in a preparative step compatible with all existing analytical steps (e.g. NGS,ddPCR, etc.).

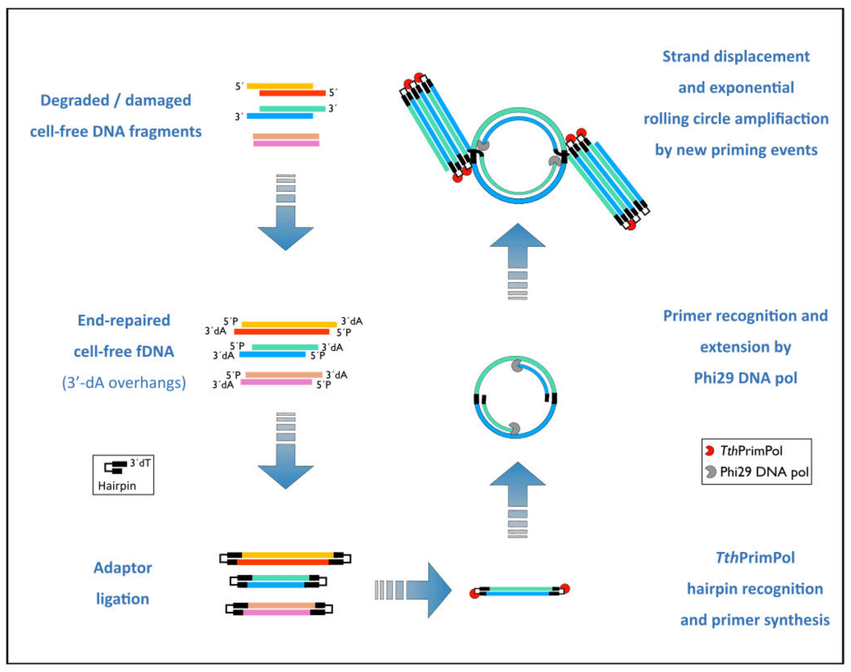
Conclusions

One of the main challenges for cell-free DNA analysis is the limited amount obtained from any bodily fluid, which affects the number and type of tests that can be performed. The TruePrime™ Apoptotic Cell-Free DNA Amplification Kit provides the solution to this issue by exponentially amplifying cell-free DNA derived from apoptosis cell-death mechanism (160-170 bp).

The TruePrime™ Apoptotic Cell-Free DNA Amplification Kit combines the novel TruePrime™ DNA amplification technology, with key novel steps of cell-free DNA pre-treatment, composed of an end-repair + dA tailing reaction and ligation of hairpin-adaptors, which enables the efficient amplification of apoptotic cell-free DNA by TruePrime™ following the rolling circle DNA amplification method.

- Excellent sensitivity - down to picograms of input material
- High amplification yields
- Flexibility, up to 150 ng or 50 µl of cfDNA input
- Error-free amplification due to the use of high-fidelity Phi29 DNA pol
- No artefacts derived from random synthetic primers
- Streamlined workflow and reduced hands-on time
- Increased mutation detection

Technology scheme



Protocol:

1. End-repair + dA tailing reaction:
- 30' 20°C and 30' 65°C
- Sample volume: 50µl
- Reaction volume: 60µl

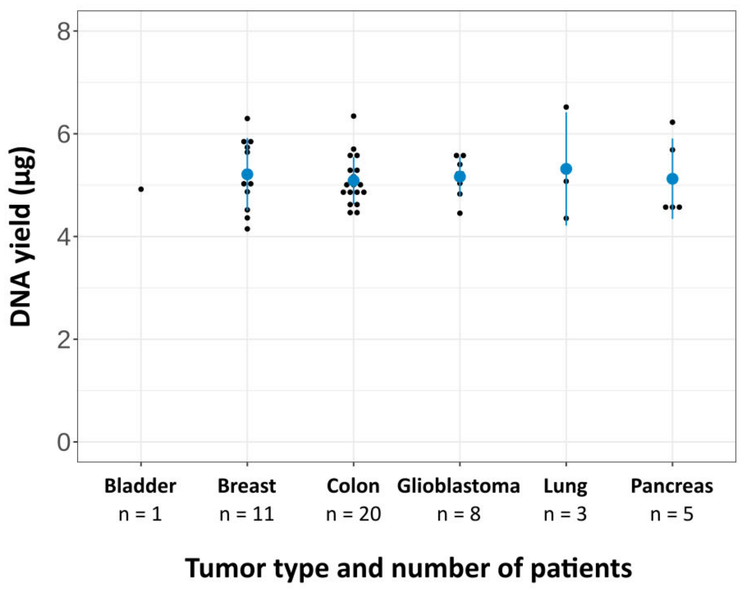
2. Adaptor ligation:
- 15' 20°C
- Sample volume: 60µl
- Reaction volume: 93.5µl

3. Cleanup of adaptor-ligated DNA:
- 30'
- Elution volume: 15µl

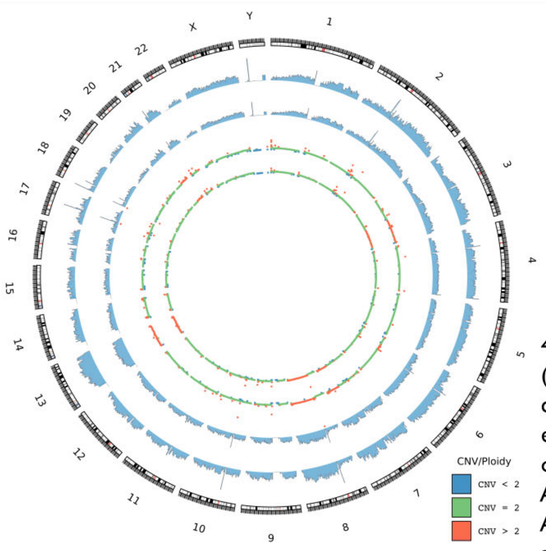
4. TruePrime amplification:
- 3h 30°C and 10' 65°C
- Sample volume: 15µl
- Reaction volume: 50µl

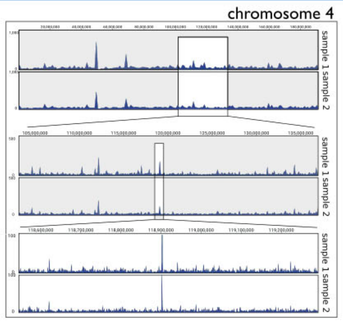
DNA yield for several tumor samples

48 patients were recruited and 10 mls of blood were extracted using Streck Cell-free DNA BCT® tubes. 3 mls of plasma were immediately isolated through a double-spin centrifugation protocol to avoid genomic DNA contamination from nucleated blood cells. Cell-free DNA was purified from 1 ml of plasma using Qiagen QIAamp® Circulating Nucleic Acid Kit. Cell-free DNA was quantified using Qubit™. 1 ng of each cell-free DNA sample was subjected to the TruePrime™ apoptotic cell-free DNA amplification workflow. The figure shows the DNA amplification yields obtained in each case. All cell-free DNA samples were efficiently amplified, producing enough DNA for any subsequent analysis or technique.



Illumina - WGS



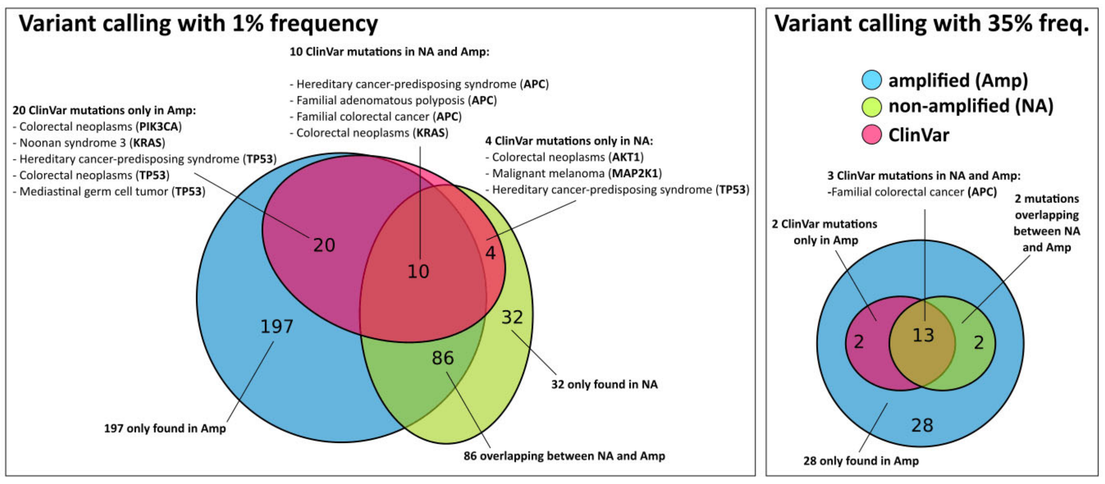


4 ng of cell-free DNA from a colon cancer patient (T3N1aM0) were subjected to the TruePrime™ apoptotic cell-free DNA amplification workflow in duplicate. 1000 ng of each amplified cell-free DNA were sheared with Covaris to obtain 500 bp fragments. Sheared DNA was purified using AMPure beads and the library was prepared using the NxSeq AmpFREE Low DNA Library Kit (Lucigen). Dual indices were added by PCR and the samples were sequenced in an Illumina HiSeq 2500 2x 150 bp.

TOP LEFT: Circos plot showing the coverage and CNV detection results. The coverage of two samples from the same patient looks nearly identical and highly even, as does the CNV plot in which except for single parts both samples have an identical composition of ploidities. The reads were analyzed by CLCGenomicsWorkbench, combined into one read and then separated again at the position of the hairpin. The hairpin sequence could be found in almost all combined reads. The again separated and artificially created new paired reads were aligned to the human genome and analyzed for the coverage statistics, reproducibility and CNV content

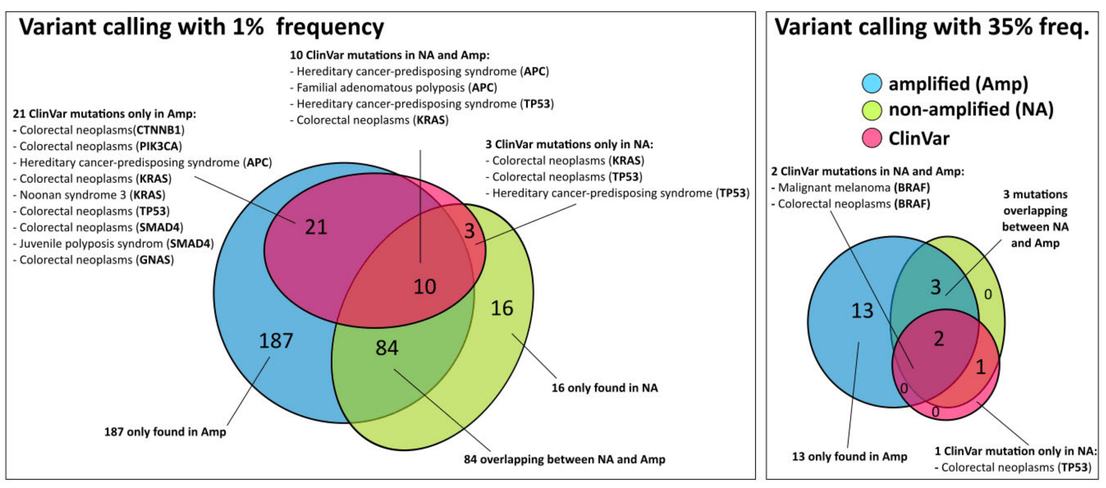
TOP RIGHT: Coverage plot from the two samples from one patient in different resolutions showing an almost identical amplification and results from the post-sequencing process.

Ion Torrent - Targeted sequencing



26.7, 84 and 150 ng of cell-free DNA from three different colon cancer patients (T3/4) were subjected to the TruePrime™ apoptotic cell-free DNA amplification workflow, obtaining 15, 17 and 20 µgs respectively. 20 ng of the non-amplified cell-free DNA and 50 ng of the TruePrime™-amplified cell-free DNA from each patient were sequenced using the Oncomine™ Colon cfDNA Assay with tag molecular barcodes for multiplexing in an Ion Proton™ sequencing system, using an Ion Proton™ Chip. Libraries were prepared using the Ion Chef™ system. Read alignment was carried out using the Torrent Suite Software and the variant calling was performed using the CLC software with the following settings: Ploidy = 2, Ignore positions with coverage above = 1000000, Restrict calling to target regions = Oncomine_Colon_cfDNA.03062017. Designed_BED, Ignore broken pairs = No, Ignore non-specific matches = Reads, Minimum coverage = 10, Minimum count = 2, Minimum frequency (%) = 1.0-35.0, Base quality filter = No, Read direction filter = No, Relative read direction filter = No, Read position filter = No, Remove pyro-error variants = No, Create track = Yes, Create annotated table = No.

Ion Torrent - Targeted sequencing



Ion Torrent - Targeted sequencing